[Continued on next page]

(54) Title: AN AUTOMATED SAMPLE PROCESSING APPARATUS AND A METHOD OF AUTOMATED TREATING OF SAMPLES AND USE OF SUCH APPARATUS
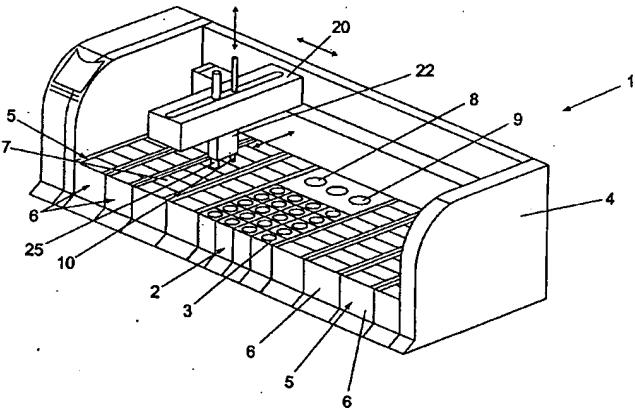
(57) Abstract: An automated staining apparatus (1) and a method for treating samples arranged on carrier means (7) is disclosed, in which portions of two or more selected reagents from a first station (2) containing the reagents are collected individually by means of a robot arm (20) handling a probe (10) and dispensed in a mixing station (9) of the apparatus. After mixing the reagents in the mixing station (9), e.g. by shaking or rotating the mixing cup containing the reagents, the reagent mixture is applied to selected samples by means of the robot arm (20) and a probe (10). By providing a staining apparatus having an automated mixer integrated therein, staining or treatment processes requiring a mixture of unmixable reagents, such as a water based and an oil based reagent, or insoluble reagents, can be performed automatically instead of requiring human interaction or manual performance of some process steps in a much more automated process, and the quality of the staining process may be improved as a desired degree of mixing of reagents may be provided or an optimal application time window for a deteriorating mixture may be reached.

An automated sample processing apparatus and a method of automated treating of samples and use of such apparatus

FIELD OF INVENTION

5   This application relates to the field of sample processing systems and methods of processing samples. The present invention may be directed to the automated processing, treatment, or even staining of samples arranged on carriers, such as slides, and in some embodiments, directed to the continuous or batch processing of samples and carriers, as well as washing elements of a sampling system.
10   Embodiments may further relate to control systems for sample processing and data acquisition, data maintenance, and data retrieval for sample processing. Applications to which the present invention may especially relate include immunohistochemistry, in-situ hybridization, fluorescent in-situ hybridization, special staining, and cytology, as well as potentially other chemical and biological
15   applications.

More specifically the present invention relates to an automated sample processing apparatus for treating samples arranged on carrier means, such as microscope slides or sample tubes, located at defined positions close to or in the apparatus by
20   aspirating a portion of selected reagent from a station containing a plurality of reagents and thereafter dispense the reagent to a sample, e.g. a tissue, organic cells, bacteria etc., arranged on the carrier means. The invention facilitates that two or more reagents are mixed and the mixture applied to a sample.

25   The invention also relates to a method of automated treating of samples by mixing reagents and dispensing the mixture to the sample.

A sample is in this application to be understood as a biological sample such as histological samples, e.g. tissue and cell specimens, including cell lines, proteins
30   and synthetic peptides, tissues, cell preparations, blood, bodily fluids, bone marrow, cytology specimens, blood smears, thin-layer preparations, and micro arrays, and specifically biological samples on microscope slides.

BACKGROUND OF INVENTION

Sample processing in immunohistochemical (IHC) applications and in other
chemical and biological analyses may require one or a number of various
processing sequences or protocols as part of an analysis of one or more samples.
The sample processing sequences or protocols may be defined by the individual or
organization requesting an analysis, such as a pathologist or histologist of a
hospital, and may be further defined by the dictates of a particular analysis to be
performed.

In preparation for sample analysis, a biological sample may be acquired by known
sample acquisition techniques and may comprise, for example in IHC applications,
tissues generally or even in some applications one or a plurality of isolated cells,
such as in microarray samples, and may be presented on a sample carrier such as
a microscope slide. Furthermore, the sample may be presented on the carrier
variously and potentially in some form of preservation. As one example, a sample
such as a layer or slice of skin may be preserved in formaldehyde and presented on
a carrier with one or more paraffin or other chemical layers infiltrating the sample.

Immunologic and histological applications, for example, may require processing
sequences or protocols that comprise steps such as deparaffinization, target
retrieval, and staining, especially for in-situ hybridization (ISH) techniques.
Previously, in some applications, these steps may have been performed manually,
potentially creating a time-intensive protocol and necessitating personnel to be
actively involved in the sample processing. Attempts have been made to automate
sample processing to address the need for expedient sample processing and a less
manually burdensome operation. However, such previous efforts may have not fully
addressed the needs for an automated sample processing system. Previous efforts
to automate sample processing may be deficient in several aspects that prevent
more robust automated sample processing, such as: the lack of sufficient computer
control and monitoring of sample processing; the lack of information sharing for
processing protocol and processing status, especially for individual samples; the

lack of diagnostic capabilities; and the lack of real-time or adaptive capabilities for multiple sample batch processing.

Past efforts at automated sample processing for samples presented on carriers such
5 as slides, such as US Patent No. 6352861 to Ventana Medical Systems, Inc. and US Patent No. 5839091 to LabVision Corporation, have not afforded the various advantages and other

Sample processing apparatuses for staining and treating samples by means of
10 probes normally comprises a first station for containing one or more reagent vials; a second station for mounting slides, a probe arranged to aspirate a portion of reagent from a selected reagent vial and dispensing the reagent to a slide on which the sample is arranged and a drive means for moving the probe between the various stations.
15

US-A-5,948,359 discloses an apparatus of the above mentioned type, wherein the first station comprises a vial holder for holding 40 or more vials in order to provide a wide range of different reagents adapted for different staining purposes, and thereby the possibility of automatically staining a large number of slides requiring different
20 staining processes. In practise it is very important that the apparatus facilitates that many different staining processes can be performed at the same time in the apparatus, because this avoids the necessity of batching samples requiring the same procedure or other treatment with reagents, and processing each batch individually.
25

US-A-5,723,092 discloses a sample dilution well for an immunoassay analyser in which a sample and a diluent is mixed by rotating the sample dilution well. Samples such as plasma or urine are loaded into the immunoassay analyser in a number of sample containing tubes, and a probe is arranged for collecting a portion of the
30 sample in a selected sample carrying tube and transferring the sample to the dilution well, adding a portion of diluent, such as water to the sample portion and mixing the two by rotating the dilution well, after which a portion of the diluted sample is aspirated from the dilution well for examination.

The mixing of reagents is known from US-A-5,425,918 disclosing a staining apparatus for treating samples arranged on carrier means, comprising a vial station for containing at least two reagent vials, a carrier means station arranged for

5 intermediate storage of a plurality of carrier means, probe drive means arranged for moving a probe, wherein the probe drive means is arranged to aspirate a portion of reagent from a selected reagent vial of the vial station by means of a probe and to apply reagent to a selected carrier means of the carrier means station. In this document it is suggested how to solved the problem regarding agents requiring

10 mixing just prior to use due to short active lifetimes after mixing. The agents may be mixed by use of the probe and probe drive means drawing up one agent from its storage vial and dispensing it into the storage vial of the second agent. The mixed solution is then drawn up and dispensed onto the slides.

15 Mixing wells for mixing samples and diluents are also disclosed by US-A-4,323,537, in which an analysis system is described having mixing cuvettes for mixing sample and diluents by rotation and a displaceable probe for collecting the raw sample from a sample container and deliver it to the mixing cuvette, and for collecting the diluted sample from the cuvette after mixing and deliver it to a plurality of analysis stations

20 for further, automated analysis.

Methods for mixing reagents and liquids are well known – but several aspects are important in the context of IHC and ISH instruments wherein the mixing in some processes can be cumbersome.

25

The staining procedure is laborious and uses many different reagents. The staining protocol may include the following steps: deparaffination, washing, antigen retrieval, endogenous biotin or enzyme blocking, incubation with immunological reagents, molecular probes, secondary visualization reagents and various chromogen

30 reagents, washing steps and counterstaining.

An on-board mixing device should be able to mix a multiple of reagents and mixtures. Non-limiting examples include:

Dilution of chromogens concentrates, mixing and dilution of two, three or four component enzyme chromogen reagents, dilution of buffer concentrates, dilution of immunological reagents with dilution buffer, dilution of visualization reagents with

5 dilution buffers, mixing several visualization reagents with dilution buffer or dilution of enzyme blocking reagents, dilution of biotin blocking reagents or mixing and dilution of counterstaining reagents.

During the mixing several problems may arise. Some problems arise due to the

10 complex use of reagents in the staining procedure. Below a few non-limiting examples are listed in more detail:

The chromogen reagents (e.g. DAB, AEC, fast red etc) often comes as concentrated reagents in organic or high viscosity solutions and needs to be diluted prior to being

15 applied to the sample.

Chromogens like the Fast Red alkaline phosphatase chromogen are made ready for use by mixing and dilution of two or three reagents, which are very different in nature with regard to salt content, viscosity and density. Furthermore, the resulting

20 mixtures are unstable over time and need to be used within a short time.

Some chromogens suitable for e.g. horseradish peroxidase, like DAB and AEC, are easily oxidized when exposed to air during e.g. vigorous mixing or dilution.

25 The enzyme chromogens and counterstain reagents like e.g. hematoxylin, are semi oxidized and can contain precipitates and solids. By further oxidation or slight change in pH, the reagents can further precipitate.

Antibody and enzyme containing reagents often contain stabilizing proteins and or

30 detergents, which causes the solution to foam when being shaken or stirred. Many proteins cannot easily tolerate to be exposed to the hydrophobic air in foam.

Wash buffers can contain detergents, which can foam when shaken or stirred. The foam can spread to other compartments of the instrument in an unwanted and unpredictable way.

5   Mixing of some reagents like e.g. the HRP chromogens and peroxide reagents can result in the formation of small bobbles. These can generate foam or bubbling on the surface of the mixture.

Spill over/carry over must be avoided. The staining process is characterized by 10   using many, complex and very different reagents and buffers and in many different dilution ratios and mixtures. Some of the reagents or buffers are incompatible with each other's. In the event of cross contamination due to e.g. carry over, the reagents may be ruined within seconds or solids can precipitate, making the staining unsuccessful. For example, enzyme containing reagents can not be mixed with the 15   corresponding chromogens, or high salt concentrates may not be mixed with e.g. proteins containing mixtures, or organic solvents can not be mixed with protein containing mixtures, or highly pH buffered wash buffers can not be mixed with low buffered mixtures without significantly altering the properties of the reagents. Accordingly the cleansing and washing of the mixing device need to be very 20   efficient.

As the procedures are very complex, and the instrument uses many different protocols, one cannot predict the result of reagent carry-over or unplanned mixing of reagents. Consequently, the mixing device should ideally be very efficient and be 25   designed for a variety of reagent mixing protocols and sequences.

Build-up of small fouling layers on the surfaces will fast cause problems, as the typical staining protocol calls for many mixing and dilution steps. Consequently, the mixing device should have a minimum of surface area and very smooth surfaces.

30

Furthermore, the mixing device should ideally be able to mix very different volumes of reagents in both small and large volumes ratios. In other words, the degree of dilution and mixing ratios of reagents may vary from small to high ratios.

In summary, the mixing device should ideally allow:

- Mixing of small and large volumes
5 - Mixing reagents with different viscosities and densities
- Mixing of immiscible or nearly immiscible reagents
- No fouling of mixing rods or similar due to precipitated material
- Allow easy escape of formed gasses during mixing
- Prevent foaming of e.g. detergent or protein containing reagents
10 - Low build-up of debris or fouling on the device surfaces
- Easy emptying and washing – regardless of volumes
- Very low reagent carry-over

No present mixing system truly fulfils the above-mentioned properties. On the slide
15 mixing does not allow for very large ratios of dilution. Nor does it allow for efficient mixing of reagents with very different densities or viscosity.

The object of the present invention is to improve the known apparatuses for sample processing as well as the method for automatic sample processing by facilitating a
20 wider range of available processes of treatment, so as to increase the number of different staining and/or treatment processes that may be performed automatically, alternatively or additionally to provide an increased quality of some specific staining processes.

25 SUMMARY OF INVENTION
This is achieved by the sample processing apparatus according to the present invention, comprising a reagent mixer having
a mixing cup for receiving two or more reagents and mixing means for mixing the reagents in the mixing cup, and
30 means for dispensing the reagent mixture from the mixing cup to a selected carrier means.

An apparatus according to the invention may comprise an automated sample processing system comprising a plurality of drawers, a plurality of sample carrier retainment assemblies each removably configured with one of the drawers, and an adaptive sample processing control system to which the drawers and the sample

5   carrier retainment assemblies are responsive. The sample carrier retainment assemblies may comprise slide retainment assembly and may be removably configured with the drawers to provide sample processing with the drawers of the system. The adaptive sample processing control system may automate the sample processing system such that one or more batches of samples may be processed

10   according to one or more protocols, potentially indicated by information on the slides that may be automatically identified by the adaptive sample processing control system. Sample processing may comprise one or more sampling protocols and steps, such as deparaffinization, target retrieval, and staining.

15   The term staining is used for the end product of the process, by which certain parts of the sample may be stained, i.e. have obtain a different colour, either in the optic range or in another electromagnetic range, such as ultra violet, or the staining may be an detectable, preferably automatically detectable, change in properties, such as fluorescent properties, magnetic properties, electrical properties or radioactive

20   properties. To obtain the staining, the sample normally have to undergo a series of treatment steps, such as - but not limited to - washing, binding of reagents to the specific parts of the sample, activation of the reagents, etc. and each treatment step may include a plurality of individual treatments.

25   In some staining processes, it is required for one or more treatments to use a mixture of reagents prepared from two or more separate reagents which may be somewhat incompatible e.g. unmixable, such as a water based and an oil based reagent, or insoluble, and therefore requires that the two or more reagents are manually prepared and introduced into a reagent vial shortly before starting the

30   staining process in order to obtain the best possible staining result for the selected examination purposes. For other processes, different staining process steps require a mixture of the same two reagents but in different dissolution ratios. Some process step may require a mixture of two or more reagents that, when mixed, becomes

unstable, e.g. has a limited time window of usability because internal chemical processes deteriorates the mixture. By providing a sample processing apparatus having an automated mixer integrated therein, these types of staining processes can be performed automatically instead of requiring human interaction or manual

5   performance of some process steps, thereby achieving a much more automated process, and the quality of the staining process may be improved as a desired degree of mixing of reagents may be provided as well as an optimal application time window for a deteriorating mixture may be reached.

10   Thus, the present invention relates to a sample processing apparatus for treating samples arranged on carrier means, comprising
a vial station for containing at least two reagent vials,
a carrier means station arranged for intermediate storage of a plurality of carrier means,

15   probe drive means arranged for moving a probe,
wherein the probe drive means is arranged to aspirate a portion of reagent from a selected reagent vial of the vial station by means of a probe and to apply reagent to a selected carrier means of the carrier means station,
wherein the apparatus comprises a mixing station with a reagent mixer having

20   a mixing cup for receiving two or more reagents and mixing means for mixing the reagents in the mixing cup, and
means for dispensing the reagent mixture from the mixing cup to a selected carrier means of the carrier means station.

25   The vial station is a collection of a plurality of vials, at least two, but often 20-60 vials or more, which may or may not be physically arranged in close proximity to each other. The term station does not indicate that the vials must be located within one, confined area; rather it indicates the existence of a plurality of vials. The probe drive means may be a robot arm with two or three degrees of freedom, such as an

30   articulated arm or one track or a set of perpendicular tracks along which a probe retainer of the probe drive means may be displaced, wherein the probe retainer may be moved in a direction normal to the track or tracks. The skilled person may readily design other types of probe drive means, e.g. combinations of the above described.

The carrier means may be provided to the apparatus in a two-dimensional array, e.g. constituted by individual rows of carrier means as discussed in the example below, or the carrier means may be provided in any manner known in the art, e.g. arranged in a carrousel or as a row of carrier means. The carrier means may also be

5 arranged movably with respect to the probe drive means, such as in an endless row that is advanced automatically past the operating area of the probe drive means or as a two-dimensional array that may be moved in a direction perpendicular to a travel direction of the probe drive means, so that the probe may reach any carrier means by the combined movement of the probe and the array.

10

The carrier means are preferably arranged in groups or series on trays or the like, so that a plurality of carrier means may be removed from or inserted into the apparatus simultaneously, and the apparatus preferably also comprises means for performing the intermediate storage of the carrier means with samples thereon and

15 the removal of the carrier means from the apparatus automatically.

The operation of the sample processing apparatus will generally be controlled by means of control means, typically a computer having a central processing unit and one or more memory unit associated therewith, means for controlling the various

20 operations of the apparatus by controlling step motors, solenoids, valves and/or other drive or control parts of the apparatus. The control means may have one or more data communication ports for enabling data communication with external computers by wire or wireless. The control means does not have to be physically arranged within the apparatus itself but may be a computer external to the sample

25 processing apparatus and connected to the apparatus via a data transmission port thereof.

It is advantageous that the probe drive means is arranged to apply the reagent mixture from the mixing cup to selected carrier means. The mixed reagents from the

30 mixing cup may be applied to the samples by separate means, such as a separate probe and probe drive means. In an alternative embodiment the carrier with the sample in question may be moved to a location at an outlet from the mixing cup. However it is presently preferred to use the probe drive means for the task.

The mixing cup may receive the two or more reagents to be mixed from a separate set of vials or other reagent sources, but it is for rationalisation of the apparatus preferable that at least some of the reagents to be mixed may come from the same vials as are used for containing reagents to be applied to the samples. In a preferred embodiment, the probe drive means is therefore arranged to aspirate portions of reagents from at least two selected reagent vials of the vial station and apply said portions of reagents to the mixing cup.

The mixing means of the reagent mixer may advantageously be constituted by cup drive means arranged for cyclic movement of the mixing cup, e.g. shaking or rotation in a horizontal or a vertical plane, so as to mix reagents contained in the mixing cup. The mixing of the reagent may be further improved by arranging mixing elements, such as blades or edges within the cup and stationary with respect to the cup. Other known mixer types may instead be preferred, such as shaft-driven impellers or magnetically driven impellers.

The cyclic movement is preferably a rotation of the mixing cup, advantageously about a substantially vertical axis. The rotation is in a preferred embodiment an intermittent rotation in a clockwise and in an anticlockwise direction. Preferably the rotation speed may be varied through control means acting upon a driving motor in such manner that the fluid content inside the mixing cup is agitated, but at so low speed that substantially all reagents remain in the mixer cup. Preferably the speed may be controlled depending on the type of reagent in the mixer cup. Preferably the speed is controlled according to the processing protocol controlling the processing of the sample(s) to be processed in the apparatus. Preferably the rotation pattern (speed and direction) is controlled by controlling means, such as a computer, according to the sample processing protocol, thereby optimising the quality the mixing in the mixer cup in respect to the reagents present in the mixer cup. For the sake of good order, by sample processing protocol is meant a sequence of processing steps defined for the actual sample treated in the sample processing apparatus.

In order to provide an efficient and fully automated cleansing of the mixing cup when changing from one reagent mixture to the next, it is advantageous that the mixing cup has inner walls extending upwardly and outwardly from a bottom part of the mixing cup, and the cup drive means is capable of rotating the mixing cup at an angular speed sufficient to fling all reagents contained therein out of the mixing cup. Preferably the inner walls are smooth and may terminate in an upper rim at the widest inner diameter of the mixing cup, in which case the high-speed rotation, preferably about a symmetry axis of the cup, will cause the waste reagent or cleansing liquid to be flung out over this upper rim. In an alternative embodiment of the mixer, the inner walls of the cup extend upwards and inwardly above the level of the widest inner diameter, at which one or more exit openings are provided in the inner wall as outlets for the waste reagent or the cleansing liquid. The top may be open for the probe to enter the mixing cup, or it may be closed to facilitate that the cyclic movement of the mixing cup for mixing the reagents may have a vertical component. The probe may in this case enter the mixing cup through one of the exit openings.

To collect the waste reagent or the cleansing liquid, it is preferred that the reagent mixer comprises a waste reagent collecting chamber having a sidewall part laterally surrounding the mixing cup and arranged to collect reagents flung out of the mixing cup by rotation of the cup.

In a further preferred embodiment, the reagent mixer further comprises a mixing cup holder for receiving and supporting the mixing cup in a releasable manner, so that different mixing cups may be used for different reagent mixtures.

In yet further preferred embodiment, replacing means are provided for replacing the probe that is moved by the probe drive means with another probe, so as to avoid contamination of the reagents in the various reagent vials by the use of more than one probe. This may be combined with a probe washing station, in which one probe is washed after it has been replaced at the probe drive means so that it is clean and ready for repeated use without risking contamination of the content of the reagent vials.

The present invention also relates to a method of fully automated treating of samples arranged on carrier means by means of a sample processing apparatus controlled by means of a control means, wherein the method comprises the steps of:

5   arranging a plurality of carrier means intermediately in a carrier means station, each carrier means having a sample arranged thereon,

dispensing a portion of a first reagent selected from a plurality of reagents to a mixing cup,

dispensing a portion of a second reagent selected from a plurality of reagents to the mixing cup,

10   to the mixing cup,

mixing the reagents in the mixing cup by means of mixing means,

moving a probe to the mixing cup by means of a probe drive means,

aspirating a portion of the mixed reagents from the mixing cup by means of the probe,

15   moving the probe to a selected one of said carrier means, and

dispensing the mixed reagents to the selected carrier means,

so as to perform a treatment of the sample arranged on the selected carrier means.

In a preferred embodiment, the steps of dispensing portions of the first and the

20   second reagents comprises the steps of

moving a probe to a first, selected reagent vial by means of the probe drive means,

aspirating a portion of the first reagent from the first reagent vial by means of the probe,

25   moving the probe to the mixing cup by means of the probe drive means,

dispensing the portion of the first reagent to the mixing cup,

moving a probe to a second, selected reagent vial by means of the probe drive means,

aspirating a portion of the second reagent from the second reagent vial by means of

30   the probe,

moving the probe to the mixing cup by means of the probe drive means, and

dispensing the portion of the second reagent to the mixing cup.

In a further preferred embodiment of the present invention, the method further comprises the step of replacing the probe arranged with the probe drive means with another probe between handling two different reagents or between handling a reagent and a reagent mixture.

5

The present invention further relates to the use of an apparatus of the present invention as described above for exercising the method of the present invention. More specifically the invention relates to the use of the apparatus for IHC and ISH and sample processing of the type using special stains as well as batch-mode

10   sample processing.

Brief description of the figures
The invention will now be explained in further detail with reference to the enclosed drawing, wherein

15

Figure 1 is a perspective view of a sample processing apparatus according to the invention,
Figure 2 is a plan view of a sample processing apparatus according to the invention,
Figure 3 is a perspective view of a detail of the sample processing apparatus

20   according to figure 2,
Figure 4 is a perspective view of a reagent mixer according to the invention, and
Figure 5 is a vertical cross-section of the reagent mixer according to figure 4.

The embodiment shown in the figures and described in details below is only an

25   example of an apparatus in accordance with the present invention and is not limiting the wider scope of the invention as described in the enclosed claims.

Detailed description of preferred embodiments of the invention
Figure 1 shows one schematic embodiment of a sample processing system 1 in

30   accordance with the present invention. Cabinet sections 4 form outer portions of the system and serve to address general structural considerations of the system (a top cabinet section is not shown in Figure 1). The sample processing system may comprise a plurality of drawers 6 used for the handling and processing of samples

and sample carriers such as slides, potentially microscope slides. Other sample carriers may be accommodated consistent with the present invention. Each drawer may be configured to accommodate sample carrier retainment assemblies, such as slide retainment assemblies, carrier racks, modules, or magazines.

5

One embodiment of a sample carrier retainment assembly may comprise a slide retainment assembly. The slide retainment assembly may comprise a slide rack, module, or magazines. Slide retainment assembly is configured to accommodate a plurality of slides in at least one configuration in corresponding sample carrier retention devices. The sample carrier retainment assemblies, are utilized in the

10 processing of samples as further described below. It should be further noted that the sample carrier retainment assembly can be removably configured with the drawers, and may be stackable or nested within other retainment assemblies.

15 One or more drawers may be provided to accommodate processing materials such as reagent containers 3 for sample processing, also further described below. A processing material retainment assembly, such as a container rack may be utilized to accommodate reagent containers or other processing materials within each of drawers 110. Bottle inserts may be preferably configured with the retainment

20 assembly to ensure proper processing material positioning within the processing material retainment assembly and the drawer.

Multiple drawers 6 allow for one or a plurality of sample processing protocols to be performed by the system 1. Past efforts at sample processing, as previously

25 described, may have been limited to processing sequences for an entire batch of carriers within the system. The present invention, however, in part by providing a plurality of drawers and carrier retainment assemblies, may allow for multiple batch processing, including real-time or adaptive capabilities for multiple batch processing, as further described below.

30 The system may access, use and wash multiple probes or syringes for pipetting or otherwise aspirating fluids required for the staining of samples mounted or otherwise presented on slides. To eliminate cross contamination a system with a single reusable probe may wash the probe between each fluid applied. The task of

washing the probe can have a large impact on the throughput of the overall system. The present invention may allow for multiple probes to be available to the system for use. The system may continuously have a clean, uncontaminated, sterilized, or an unused probe available to use and sample processing is not impacted by the

5 required cleaning routine. The cleaning routine may be necessary to eliminate the possible cross contamination of fluids and, in some embodiments, may take up to about 1 minute to accomplish. The cumulative impact of the cleaning routine on a series of processing steps can add time to the throughput capabilities of the system. The addition of multiple probes or syringes may eliminate this impact and

10 significantly decreases the time required to process the samples.

Embodiments of the present invention comprise a mixing station 9 (best shown in Figures 4 and 5). The system may mix component fluids, such as dyes, buffers, or other processing materials, preferably on demand and as the processing steps and

15 protocols dictate. Fluids required during the processing steps may sometimes need to be mixed with other fluids to create a final activated fluid. However, the activity levels of these mixtures can be time sensitive and may therefore only be effective for a short period of time. The on demand mixing of fluids is advantageous in that it allows the fluids to be mixed immediately before being used. The syringe or probe

20 10, in preferred embodiments, will aspirate fluids into and from the mixing station 9 to mix component fluids. A rinse may further be dispensed into the mixing station to sterilize the station.

As an example a presently preferred embodiment of a sample processing

25 apparatus, also called a staining apparatus 1, according to the invention is shown in figures 1 and 2. The sample processing apparatus 1 comprises a rectangular frame 4 surrounding a first station 2 comprising an array of compartments wherein each compartment a reagent vial 3 is placed, and a second station 5 wherein a number of separate racks 6 is placed, and where each rack comprises a number of microscope

30 slides 7 mounted side by side in the rack 6. In the embodiment shown, each rack may hold up to 8 slides, but the rack may be designed to hold any suitable number of slides. With eight racks arranged side by side, the shown embodiments may hold

up to 64 slides 7 each having a sample, e.g. a tissue mounted on the upper side of the slide, so that reagent may be applied from above to the sample on each slide.

A robot arm 20 in Figure 1 for moving a probe 10 in X and Y (as well as Z) direction as indicated by the arrows X and Y is arranged above the frame 4 of the sample processing apparatus. The robot arm may is therefore position the probe 10 above all reagent vials 3 as well as above all the microscope slides 7, and may further operate the probe 10 to aspirate portions of reagent contained in any of the vials 3, to transfer the portion of reagent and apply it to any of the slides 7 in order to provide a selected staining or treatment of the sample on each slide 7. By use of suitable control means e.g. a computer (not shown) having the appropriate software and input data for the purpose, this staining apparatus 1 is able to automatically staining or treating samples requiring different staining or treatment reagents and processes.

The same staining apparatus viewed from below the robot arm is shown in figure 2, disclosing the probe 10 being manipulated by the robot arm. The probe 10 is raised to an upper position (in a Z direction where it is clear of the vials 3 underneath the probe 10, but the robot comprises means (not shown) for lowering the probe 10 in order to dip the probe tip 11 into the content of a selected reagent vial 3 and to aspirate a selected amount of reagent for the selected staining or treatment process.

The staining apparatus 1 of the present embodiment further comprises at least one probe washing station 8 and a reagent mixer 9, and the robot arm is furthermore arranged to transfer the probe to the washing station 8 as well as to the reagent mixer 9. The reagent mixer 9 will be described in detail below with reference to figures 4 and 5.

The apparatus comprises a reagent mixer 9 having a mixing cup 13 wherein two or more selected reagents may be placed by means of the robot arm and the probe 10. The reagent mixer 9 thereby provides on-board mixing of any reagents contained in the reagent vials 3, and thereby more staining processes, e.g. staining requiring the use of mixing of insoluble reagents, or reagents which may only be effective a short

time after mixing, are facilitated to be performed automatically within the staining apparatus without the requirement of human interaction.

5   The mixer 9 comprises a mixing cup 13 for receiving reagents released from the probe 10. The mixing cup 13 is placed into a holder 15 by means of a complementary snap fitting means 16 and 17 arranged on the inside of the holder 15 and the outside of the mixing cup 13, respectively. A motor 18 is arranged for rotating the holder 15 and thereby the mixing cup 13, either intermittently clockwise and anticlockwise in order to provide a mixing of reagents contained in the mixing 10   cup, or by spinning the holder 15 and thereby the mixing cup 13 in order to fling out waste reagents or cleansing liquid from the mixing cup 13.

For the latter purpose, the mixing cup 13 is preferable provide with sidewalls 20 extending upwardly and outwardly from the bottom 19, e.g. forming a frusto-conical 15   cavity, and the mixing cup 13 has an upper rim 14 allowing the reagents to escape from the mixing cup 13 during the spinning process.

The reagent mixer 9 furthermore comprises a housing 21 having sidewalls 22 surrounding at least the rim 14 of the mixing cup 13 and thereby forming splash 20   faces for collecting any liquid flung out from the mixing cup 13. The housing also comprises a lid 23 for enclosing a space 24 surrounding the mixing cup 13 in order to avoid reagent spills outside the space 24. The lid 23 has a central opening 25 allowing reagents from the probe 10 to be dispensed into the mixing cup 13 from above the reagent mixer 9 as well as allowing the probe 10 to enter the mixing cup 25   13 for collecting the mixed reagents.

According to a preferred embodiment the housing also comprises a hose connection 27 for draining waste reagent or cleansing liquid from the space 24, and a tap 26 is arranged for dispensing cleansing liquid into the mixing cup 13 when required.
30
In an alternative embodiment a releasable connection 12 is provided between the probe 10 and the robot arm, enabling the replacement of the probe 10 held by the robot arm by placing the probe 10 in one of a number of free washing stations 8,

where it is released by the releasable connection 12, and where a new probe 10' is connected to the robot arm by means of the releasable connection 12.

5   Having the appropriate input data, the control means of the apparatus operates the robot arm to commence a staining or treatment run by firstly moving the probe to a first reagent vial 3, into which the probe tip 11 is inserted into the liquid and is aspirated into the probe 10 in an amount corresponding to the number of samples to be stained or treated, in accordance with the input data provided to the control means.

10

The probe 10 is subsequently, in a first operating mode moved by the robot arm towards the slide rack system 5 in which the slides 7 are mounted. The slides 7 are situated with the surface horizontally oriented and the probe 10 releases the required amount of reagent on the appropriate slides in accordance with the input 15   data. Alternatively, the probe 10 is in a second operating mode moved by the robot arm towards the reagent mixer 9 where it releases the reagent into the cup 13 of the reagent mixer 9, and is subsequently moved to the probe washing station 8, where the probe 10 is either washed or - in the alternative embodiment - released into a free washing station 8, and another probe 10' situated in another washing station 8' 20   is connected to the robot arm. The robot arm moves the new clean probe 10' to a second selected reagent vial 3 for collecting a selected amount of reagent from the second vial 3, and the probe 10' is thereafter by means of the robot arm moved to the reagent mixer 9, where the reagent in the probe 10' is dispensed into the cup 13 of the mixer containing the first selected reagent. The second operating mode can 25   according to the invention be commenced several times if more than two reagents are to be mixed for a specific staining or treatment process.

The reagent mixer 9 mixes the reagents in the cup 13 thereof, and a clean probe 10 picked up from the washing station 8 by the robot arm is lowered into the cup 13 of 30   the reagent mixer 9 to collect the mixed reagents, where after the robot arm moves the probe 10 towards the second station 5 containing the slides 7, at which the probe 10 releases the required amount of mixed reagent on selected slides 7 in accordance with the input data.

The robot arm with probe 10 is subsequently directed to a free washing station 8, and the probe 10 is either washed or alternatively replaced by a clean probe 10', where after the process in accordance with the first or the second operating mode may be repeated or continued with a new reagent or reagent mixture.

The present invention is highly suitable for on-board mixing in an automated cytological/IHC/ISH instrument.

It should be understood that by having an inner cup with smooth surface and no sharp edged, cleaning and washing is made easy. Other mixing devices using propellers or baffles or similar, all have large surface areas, edges and concavities, whereto reagents may adhere, whereby thorough washing becomes difficult, possibly resulting in unwanted carry-over.

By design, the shape of the cup allows debris, fines, precipitates or other solids of both high and low density to be centrifuged up and out of the cup at high rotation speeds. Advantageously the speed may be controlled by control means operating according to the processing protocol carried out for the samples present in the apparatus, i.e. if the reagents present in the mixer cup have high viscosity it may be advantageous applying a very high speed of rotation to ensure that all reagents are removed from the cup.

Also, the shape of the cup allow for the specific separation of immiscible liquid phases, by first removing the liquid with the highest density during centrifugation. It should be understood that the shape of the cup makes the mixing almost independent of the volume. The mixing action is the same for a wide range of volumes in the same cup. In contrast, the mixing action of e.g. a propeller mixer is depending on e.g. the volume of reagents, propeller size and container size.

The rotating action of the cup allows air to escape from the mixture and prevent foaming. The rotating action can suppress the formation and build-up of foam by forcing the air/liquid foam down to the liquid surface.

Preferably the rotation pattern (speed and direction) is controlled by controlling means, such as a computer, according to the sample processing protocol, thereby optimising the quality the mixing in the mixer cup in respect to the reagents present in the mixer cup, e.g. in such manner that the formation and build up of foam is 5 suppressed.

The rotating action of the cup does not allow much air from the atmosphere to be sucked down in the reagent mixture. This will limit the pollution and e.g. oxidation of air sensitive reagents. Other methods of mixing, like the action of propellers, can 10 draw air down into the liquid during mixture.

It should be understood that by changing the direction of the rotation, the mixing action could be changed from being very gentle to being very vigorous. Both changing the speed and direction of the rotation and a combination of rotations with 15 variable speed, various speeds, stops and change of direction can control the flow pattern in the cup.

Also, the cup can be covered with a neutral gas like the noble gasses, nitrogen, and carbon dioxide or similar to further protect the reagents during mixing. Preferable, 20 the protective gas is heavier than atmospheric gas. The layer of protective gas can easily be added and removed, and can cover any of the volumes the cup can hold.

By using an on board mixing device, the reagents mixing may be done in a batch-mode to make mixtures for treating multiple samples.
25
Also, the on board mixing allow for large dilution ratios and the possibility to dilute or mix reagents, using only a small portion of the mixture for treatment of samples, and discarding the rest of the mixture. This is advantageous, as some dilutions ratios are very large, and too small volumes of the concentrated reagent cannot easily be 30 accurately measured and added. Often a minimum volume of reagent to be measured is defined. The on board cup mixing design allow for a very high degree of flexibility in the staining protocol.

Claims:

1. A sample processing apparatus (1) for treating samples arranged on carrier means (7), comprising

5   a vial station (2) for containing at least two reagent vials (3),

a carrier means station (5) arranged for intermediate storage of a plurality of carrier means (7),

probe drive means arranged for moving a probe (10),

wherein the probe drive means is arranged to aspirate a portion of reagent from a

10   selected reagent vial (3) of the vial station (2) by means of a probe (10) and to apply reagent to a selected carrier means (7) of the carrier means station (5),

characterised in that

the apparatus (1) comprises a reagent mixer (9) having

a mixing cup (13) for receiving two or more reagents and mixing means for mixing

15   the reagents in the mixing cup (13), and

means (10) for dispensing the reagent mixture from the mixing cup (13) to a selected carrier means (7) of the carrier means station (5).

2. A sample processing apparatus according to claim 1, wherein the probe drive

20   means is arranged to apply the reagent mixture from the mixing cup (13) to selected carrier means (7) of the carrier means station (5).

3. A sample processing apparatus according to claim 1 or 2, wherein the probe drive means is arranged to aspirate portions of reagents from at least two selected

25   reagent vials (3) of the vial station (2) and dispense said portions of reagents into the mixing cup (13).

4. A sample processing apparatus according to any of claims 1 to 3, wherein the reagent mixer (9) further comprises cup drive means (18) arranged for cyclic

30   movement of the mixing cup (13) so as to mix reagents contained in the mixing cup (13).

5. A sample processing apparatus according to claim 4, wherein the cyclic movement is a rotation of the mixing cup (13) and/or wherein the rotation is an intermittent rotation in a clockwise and in an anticlockwise direction.

5   6. A sample processing apparatus according to claim 5, wherein the rotation take place about a substantially vertical axis.

7. A sample processing apparatus according to claim 4, wherein the movement is a combination of rotations with variable speed, various speeds, stops and change of
10   direction, wherein the rotation pattern (speed and direction) is controlled by controlling means, such as a computer, according to the sample processing protocol for the sample(s) processed in the apparatus.

8. A sample processing apparatus according to any of the preceding claims, wherein
15   the mixing cup (13) has inner walls (20) extending upwardly and outwardly from a bottom part (19) of the mixing cup (13), and the cup drive means (18) is capable of rotating the mixing cup (13) at an angular speed sufficient to fling reagents contained therein out of the mixing cup (13).

20   9. A sample processing apparatus according to claim 8, wherein the reagent mixer (9) comprises a waste reagent collecting chamber (21) having a sidewall part (22) laterally surrounding the mixing cup (13), and wherein waste reagent collecting chamber (21) is arranged to collect reagents flung out of the mixing cup (13) by rotation of the cup.
25

10. A sample processing apparatus according to any of the preceding claims, wherein the reagent mixer (9) further comprises a mixing cup holder (15) for receiving and supporting the mixing cup (13) in a releasable manner.

30   11. A method of fully automated treating of samples arranged on carrier means (7) by means of a sample processing apparatus (1) controlled by means of a control means, wherein the method is **characterised in that** it comprises the steps of

arranging a plurality of carrier means (7) intermediately in a carrier means station (5), each carrier means (7) having a sample arranged thereon,

dispensing a portion of a first reagent selected from a plurality of reagents to a mixing cup (13),

5   dispensing a portion of a second reagent selected from a plurality of reagents to the mixing cup (13),

mixing the reagents in the mixing cup (13) by means of mixing means,

moving the probe (10) to the mixing cup (13) by means of a probe drive means,

10   aspirating a portion of the mixed reagents from the mixing cup (13) by means of the probe (10),

moving the probe (10) to a selected one of said carrier means (7), and

dispensing the mixed reagents to the selected carrier means (7),

so as to perform a treatment of the sample arranged on the selected carrier means

15   (7).

12. A method according to claim 11, wherein the steps of dispense portions of the first and the second reagents comprises the steps of

moving a probe (10) to a first, selected reagent vial (3) by means of the probe

20   drive means,

aspirating a portion of the first reagent from the first reagent vial (3) by means of the probe (10),

moving the probe (10) to the mixing cup (13) by means of the probe drive means,

25   dispensing the portion of the first reagent to the mixing cup (13),

moving a probe (10') to a second, selected reagent vial (3) by means of the probe drive means,

aspirating a portion of the second reagent from the second reagent vial (3) by means of the probe (10'),

30   moving the probe (10') to the mixing cup (13) by means of the probe drive means, and

dispensing the portion of the second reagent to the mixing cup (13).

13. A method according to claim 11 or 12, further comprising the step of controlling the rotation pattern (speed and direction) according to the sample processing protocol thereby optimising the quality the mixing in the mixer cup in respect to the reagents present in the mixer cup.

5

14. A method according to claim 11 or 12, further comprising the step of adding a protective gas covering the reagent mixture in the mixer cup.

15. A method according to claim 14, wherein the protective gas is included in the
10   group comprising noble gasses, nitrogen, and carbon dioxide.

16. Use of an apparatus according to one of claims 1 to 10 for exercising the method according to one of claims 11 to 15 for immunohistochemistry and in-situ hybridization

15

17. Use of an apparatus according to one of claims 1 to 10 for exercising the method according to one of claims 11 to 15 for batch-mode sample processing

20

Fig. 1

Fig. 2
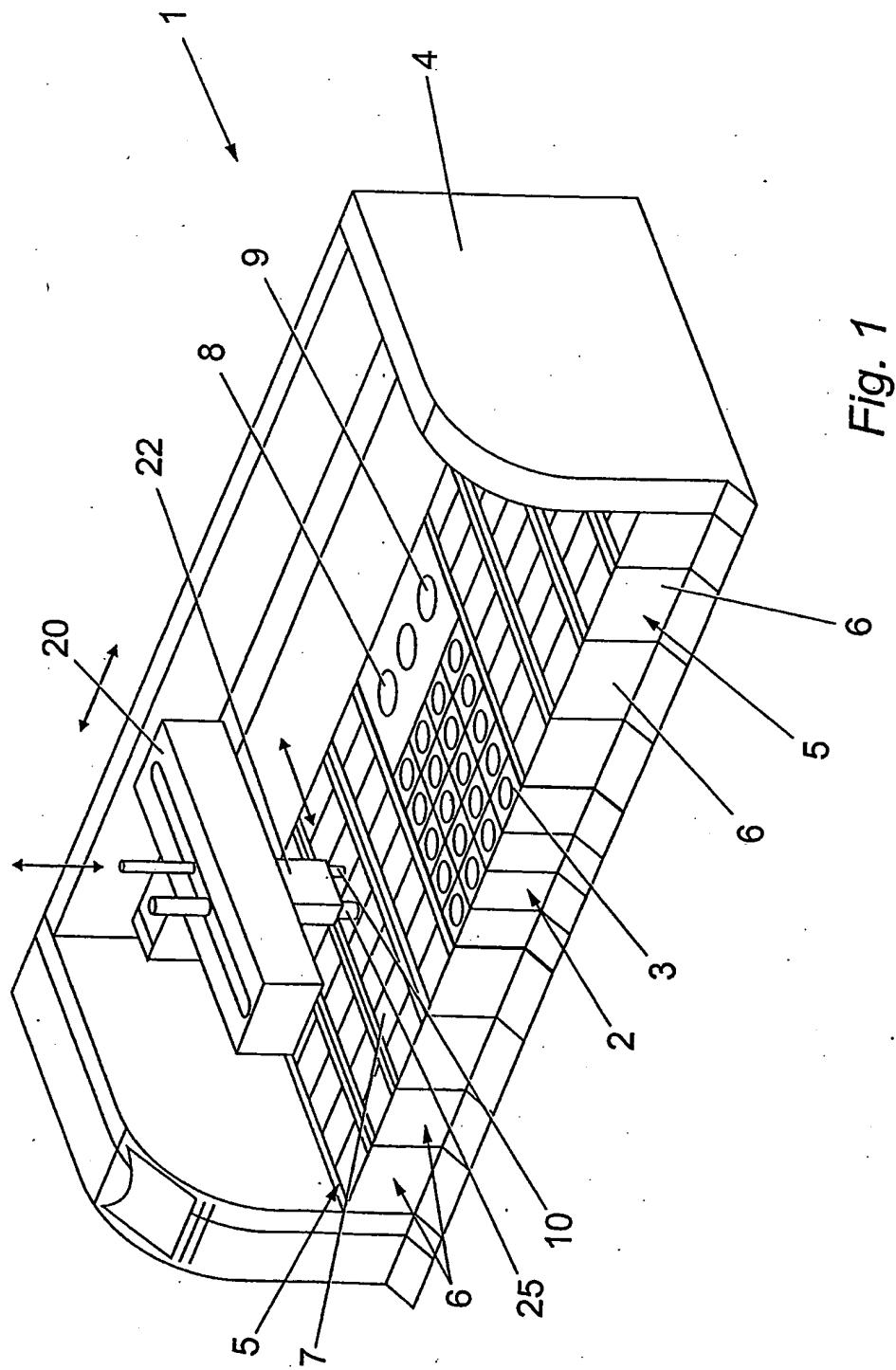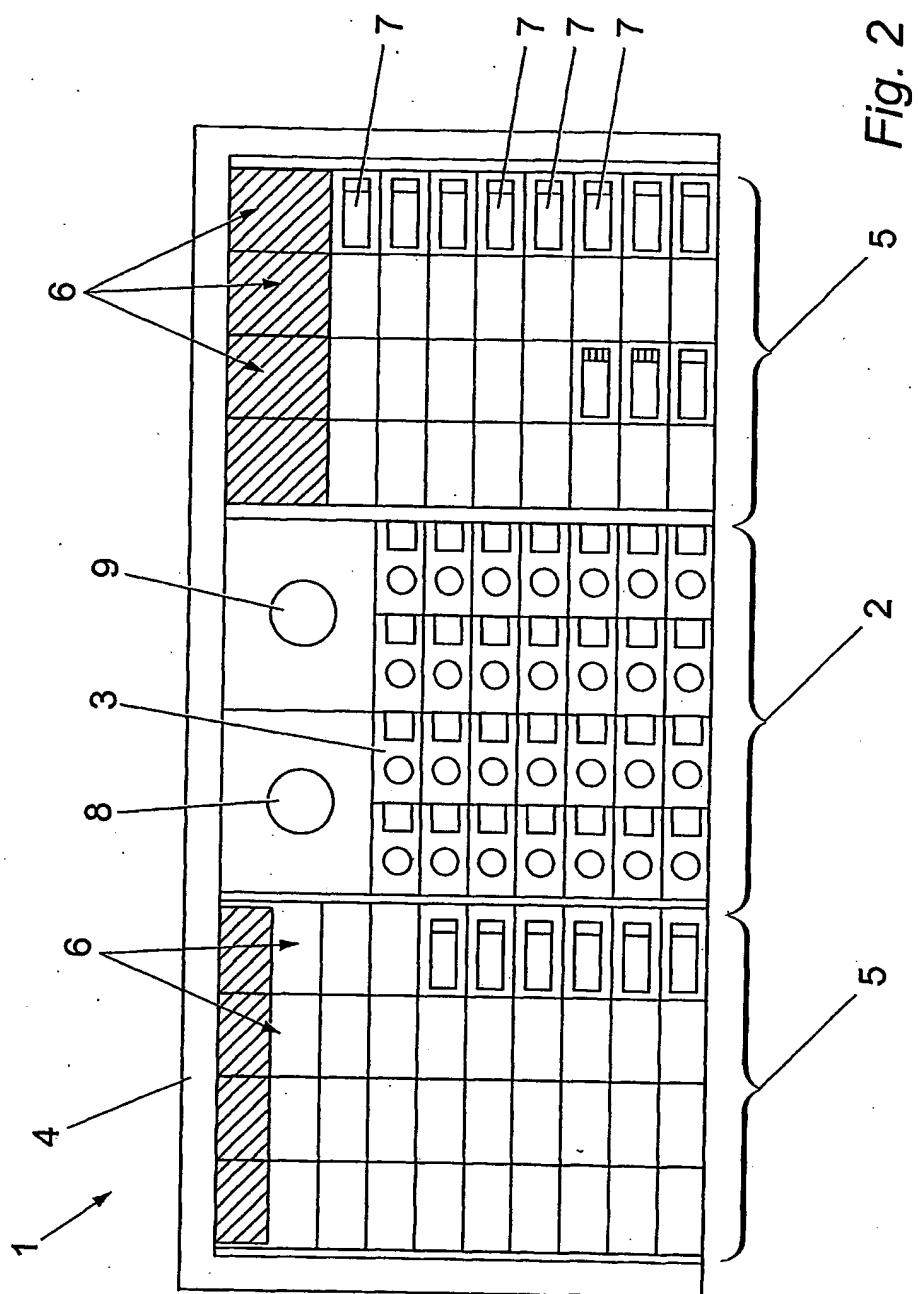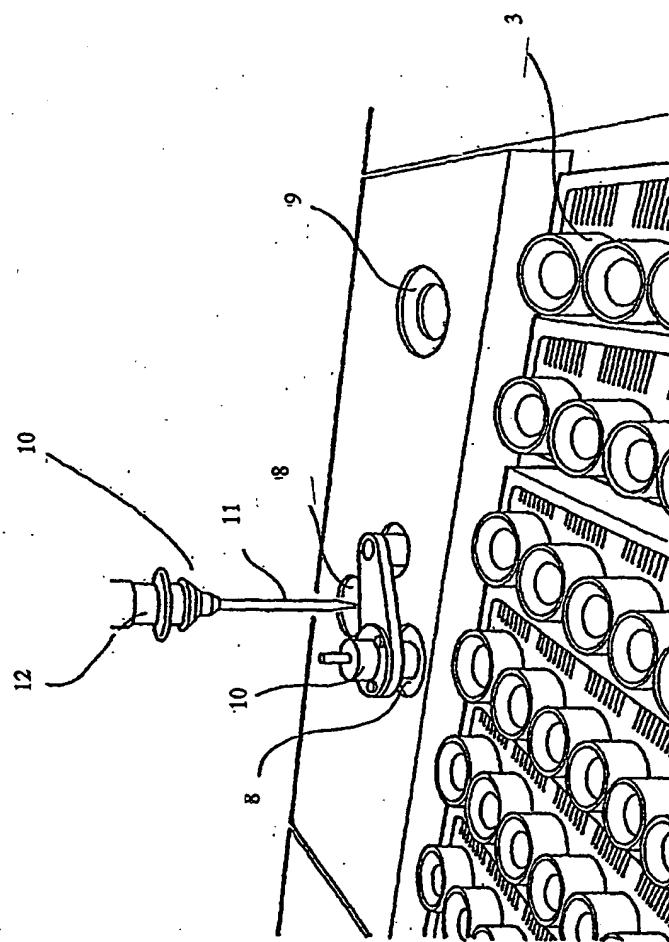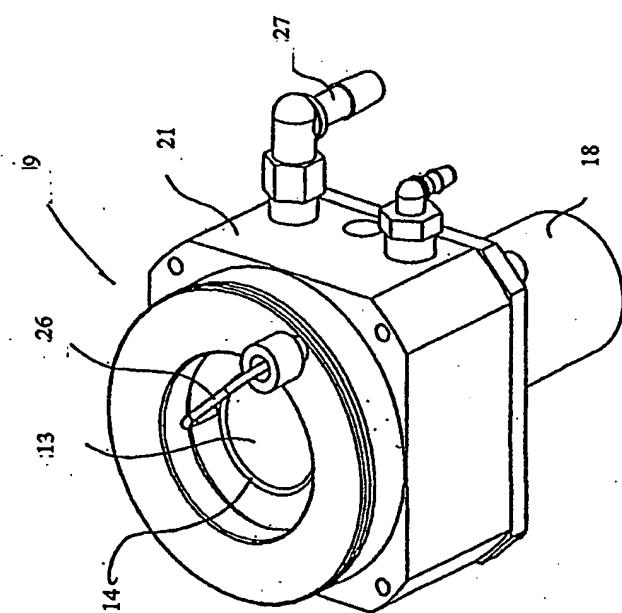
Fig. 3

Fig. 5


Fig. 4

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7   G01N1/31   G01N35/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7   G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category°	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 425 918 A (BAXTER GRAHAM L ET AL) 20 June 1995 (1995-06-20) cited in the application abstract; figures 1,2 column 3, line 47 -column 4, line 2 column 5, line 33 -column 5, line 39	1-13,16, 17
Y	US 5 723 092 A (BABSON ARTHUR L) 3 March 1998 (1998-03-03) cited in the application abstract; figure 1 column 3, line 46 -column 5, line 67	1-13,16, 17
A	US 5 948 359 A (ZHANG JASON Z ET AL) 7 September 1999 (1999-09-07) cited in the application the whole document	1-17

-/--

[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

° Special categories of cited documents :

'A' document defining the general state of the art which is not considered to be of particular relevance

'E' earlier document but published on or after the International filing date

'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

'O' document referring to an oral disclosure, use, exhibition or other means

'P' document published prior to the International filing date but later than the priority date claimed

'T' later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the Invention

'X' document of particular relevance; the claimed Invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

'Y' document of particular relevance; the claimed Invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

'&' document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the International search report
24 March 2004	15/04/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 cpo nl, Fax: (+31–70) 340–3016	Runser, C

Form PCT/ISA/210 (second sheet) (January 2004)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 839 091 A (CORL MARK V ET AL) 17 November 1998 (1998-11-17) cited in the application the whole document	1-7
A	US 6 352 861 B1 (SHOWALTER WAYNE A ET AL) 5 March 2002 (2002-03-05) cited in the application the whole document	1-17

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
US 5425918	A	20-06-1995	AU	644876 B2	23-12-1993
			AU	7754191 A	18-02-1992
			WO	9201919 A1	06-02-1992
			EP	0539379 A1	05-05-1993
			JP	6504115 T	12-05-1994
US 5723092	A	03-03-1998	AU	3582797 A	21-01-1998
			DE	19781837 T0	09-09-1999
			WO	9800697 A1	08-01-1998
US 5948359	A	07-09-1999	NONE		
US 5839091	A	17-11-1998	US	2002116132 A1	22-08-2002
			US	6349264 B1	19-02-2002
US 6352861	B1	05-03-2002	US	5654200 A	05-08-1997
			US	5595707 A	21-01-1997
			US	2002072122 A1	13-06-2002
			US	2002114733 A1	22-08-2002
			US	2002001849 A1	03-01-2002
			US	2003022391 A1	30-01-2003
			US	6472217 B1	29-10-2002
			US	5650327 A	22-07-1997
			US	5654199 A	05-08-1997
			CA	2077452 A1	03-09-1991
			CA	2299118 A1	03-09-1991
			DE	69117052 D1	21-03-1996
			DE	69117052 T2	14-11-1996
			DK	517835 T3	10-06-1996
			EP	0517835 A1	16-12-1992
			ES	2085471 T3	01-06-1996
			JP	5504627 T	15-07-1993
			JP	3186764 B2	11-07-2001
			WO	9113335 A1	05-09-1991